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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Fusion Proteins with Immunoglobulin Portions, the Preparation and Use Thereof

(72) Lauffer, Leander - Germany (Federal Republic of) ;
Oquendo, Patricia - Germany (Federal Republic of) ;
Zettlmeissl, Gerd - Germany (Federal Republic of) ;
Seed, Brian - U.S.A. ;

(73) Behringwerke Aktiengesellschaft - Germany (Federal Republic of) ;
General Hospital Corporation (The) - U.S.A. ;

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Abstract of th disclosur

Fusion proteins with immunoglobulin portions, the preparation and use thereof

The invention relates to genetically engineered soluble fusion proteins composed of human proteins not belonging to the immunoglobulin family, or of parts thereof, and of various portions of the constant region of immunoglobulin molecules. The functional properties of the two fusion partners are surprisingly retained in the fusion protein.

Behringwerke Aktiengesellschaft
and
The General Hospital Corporation

HOE 90/B 026 - Ma 824
Dr. LP

2045869

Description

Fusion proteins with immunoglobulin portions, the preparation and use thereof

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The invention relates to genetically engineered soluble fusion proteins composed of human proteins not belonging to the immunoglobulin family, or of parts thereof, and of various portions of the constant region of immunoglobulin molecules. The functional properties of the two fusion partners are, surprisingly, retained in the fusion protein.

EP-A 0 325 262 and EP-A 0 314 317 disclose corresponding fusion proteins composed of various domains of the CD4 membrane protein of human T cells and of human IgG1 portions. Some of these fusion proteins bind with the same affinity to the glycoprotein gp120 of human immunodeficiency virus as the cell-bound CD4 molecule. The CD4 molecule belongs to the immunoglobulin family and, consequently, has a very similar tertiary structure to that of immunoglobulin molecules. This also applies to the α chain of the T-cell antigen receptor, for which such fusions have also been described (Gascoigne et al., Proc. Natl. Acad. Sci. USA, vol. 84 (1987), 2937-2940). Hence, on the basis of the very similar domain structure, in this case retention of the biological activity of the two fusion partners in the fusion protein was to be expected.

The human proteins which are, according to the invention, preferably coupled to the amino terminus of the constant region of immunoglobulin do not belong to the immunoglobulin family and are to be assigned to the following classes: (i) membrane-bound proteins whose extracellular domain is wholly or partly incorporated in the fusion. These are, in particular, thromboplastin and cytokin

r c ptors and growth factor r c ptors, such as th c llular rec ptors for int rl ukin-4, int rl ukin-7, tumor necrosis factor, GM-CSP, G-CSP, erythropoietin; (ii) non-membrane-bound soluble proteins which are wholly or partly incorporated in the fusion. These are, in particularly, proteins of therapeutic interest such as, for example, erythropoietin and other cytokines and growth factors.

The fusion proteins can be prepared in known pro- and eukaryotic expression systems, but preferably in mammalian cells (for example CHO, COS and BHK cells).

The fusion proteins according to the invention are, by reason of their immunoglobulin portion, easy to purify by affinity chromatography and have improved pharmacokinetic properties in vivo.

In many cases, the Fc part in fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations.

There are in existence various proteases whose use for this purpose appears conceivable. Papain and pepsin are employed, for example, to generate F(ab) fragments from immunoglobulins (Immunology, ed. Roitt, I. et al., Gower Medical Publishing, London (1989)), but th y do not cl av in a particularly specific manner. Blood coagulation factor Xa by contrast recognis s in a pr t in th r lativ ly rar t trapeptid sequ nc Il -Glu-Gly-Arg and performs a hydrolytic cl avage of th pr tein after thc

arginin r sidu . S quenc s which c ntain th d scribed tetrapeptid wer introduc d first by Nagai and Thogersen in a hybrid protein by genetic engineering means (Nagai, K. and Thogersen, H.C., Nature, vol. 309 (1984), 810-812). These authors were able to show that the proteins expressed in E. coli actually are specifically cleaved by factor Xa. However, there is as yet no published example of the possibility of such proteins also being expressed in eukaryotic and, especially, in animal cells and, after their purification, being cleaved by factor Xa. However, expression of the proteins according to the invention in animal cells is preferable because only in a cell system of this type is there expected to be secretion of, for example, normally membrane-bound receptors as fusion partners with retention of their natural structure and thus of their biological activity. Secretion into the cell culture supernatant facilitates the subsequent straightforward purification of the fusion protein.

The invention thus relates to genetically engineered soluble fusion proteins composed of human proteins not belonging to the immunoglobulin family, or of parts thereof, and of various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as immunoglobulin is the constant part of the heavy chain of human IgG, particularly preferably of human IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed in a simple way by a cleavage sequence which is also incorporated and can be cleaved with factor Xa.

Furth rmor , th inv ntion relat s to proc ss s for th pr paration f th s fusion proteins by g n tic ngineering, and to th us th reof for diagnosis and th rapy.

The invention will now be described in relation to th drawings, in which:

Figure 1 shows two oligonucleotide probe molecules used in cloning of thromboplastin cDNA;

Figure 2 shows the nucleotide sequence of clone 2b-Apr5 with the thromboplastin amino acid sequence deduced therefrom;

Figure 3 shows two oligonucleotide sequences which are partially homologous with the sequence of the coding strand (A), and with the non-coding strand (B) of thromboplastin cDNA;

Figure 4 shows the restriction map of plasmid pTF1Fc;

Figure 5 shows two oligonucleotide sequences which are partially homologous with the sequence of the coding strand (A), and with the non-coding strand (B) of the IL-4 receptor cDNA cloned in the vector pDC302/T22-8;

Figure 6 shows the restriction map of plasmid pIL4RFc;

Figure 7 shows two oligonucleotide sequences A and B which are partially homologous with the sequence of the coding strand (A), and with the non-coding strand (B) of the EPO cDNA cloned in the vector pCES; and

Figure 8 shows the restriction map of plasmid pEPOFc.

Finally, th invention is xplained in further examples.

Example 1: Thromboplastin fusion proteins

Blood coagulation is a process of central importance in the human body. There is appropriately delicate regulation of the coagulation cascade, in which a large number of cellular factors and plasma proteins cooperate. These proteins (and their cofactors) in their entirety are called coagulation factors. The final products of the coagulation cascade are thrombin, which induces the aggregation of blood platelets, and fibrin which stabilizes the platelet thrombus. Thrombin catalyzes the formation of fibrin from fibrinogen and itself is formed by limited proteolysis of prothrombin. Activated factor X (factor Xa) is responsible for this step and, in the presence of factor Va and calcium ions, binds to platelet membranes and cleaves prothrombin.

Two ways exist for factor X to be activated, the extrinsic and the intrinsic pathway. In the intrinsic pathway a series of factors is activated by proteolysis in order for each of them to form active proteases. In the extrinsic pathway, there is increased synthesis of thromboplastin (tissue factor) by damaged cells, and it activates factor X, together with factor VIIa and calcium ions. It was formerly assumed that the activity of thromboplastin is confined to this reaction. However, the thromboplastin/VIIa complex also intervenes to activate the intrinsic pathway at the level of factor IX. Thus, a thromboplastin/VIIa complex is one of the most important physiological activators of blood coagulation.

It is therefore conceivable that thromboplastin, apart from its use as diagnostic aid (see below), can also be employed as constituent of therapeutic agents for treating inborn or acquired blood coagulation deficiencies. Examples of this are chronic hemophilias caused by a deficiency of factors VIII, IX or XI or ls acute disturbances of blood coagulation as a consequence of, for example, liver or kidney diseases. Use of such a

th rapeutic ag nt aft r surgicial int rv ntion would also be conc ivabl .

Thromboplastin is an integral membrane protein which does not belong to the immunoglobulin family. Thromboplastin cDNA sequences have been published by a total of four groups (Fisher et al., Thromb. Res., vol. 48 (1987), 89-99; Morrisey et al., Cell, vol. 50 (1987), 129-135; Scarpati et al., Biochemistry, vol. 26 (1987), 5234-5238; Spicer et al., Proc. Natl. Acad. Sci. USA, vol. 84 (1987), 5148-5152). Thromboplastin cDNA contains an open reading frame which codes for a polypeptide of 295 amino-acid residues, of which the 32 N-terminal amino acids act as signal peptide. Mature thromboplastin comprises 263 amino-acid residues and has a three-domain structure: i) amino-terminal extracellular domain (219 amino-acid residues); ii) transmembrane region (23 amino-acid residues); iii) cytoplasmic domain (carboxyl terminus; 21 amino-acid residues). In the extracellular domain there are three potential sites for N-glycosylation (Asn-X-Thr). Thromboplastin is normally glycosylated but glycosylation does not appear essential for the activity of the protein (Paborsky et al., Biochemistry, vol. 29 (1989), 8072-8077).

Thromboplastin is required as additive to plasma samples in diagnostic tests of coagulation. The coagulation status of the tested person can be found by the one-stage prothrombin clotting time determination (for example Quick's test). The thromboplastin required for diagnostic tests is currently obtained from human tissue, and the preparation process is difficult to standardize, the yield is low and considerable amounts of human starting material (placentae) must be supplied. On the other hand, it is to be xpected that pr paration of native, membrane-bound thromboplastin by g n tic ngineering will also be difficult owing to complex purification proc s-s s. Th se difficulti s can be avoided by th fusion according to th inv ntion to immunoglobulin portions.

Th thromboplastin fusion proteins according to the invention ar s cr t d by mammalian c lls (for xampl CHO, BHK, COS c lls) into the cultur medium, purified by affinity chromatography on prot in A-Sepharose and have surprisingly high activity in the one-stage prothrombin clotting time determination.

Cloning of thromboplastin cDNA

The sequence published by Scarpati et al., Biochemistry, vol. 26 (1987), 5234-5238, was used for cloning the thromboplastin cDNA. Two oligonucleotide probe molecules (see Fig. 1) were derived from this. These two probe molecules were used to screen a cDNA bank from human placenta (Grundmann et al., Proc. Natl. Acad. Sci. USA, vol. 83 (1986), 8024-8028).

cDNA clones of various lengths were obtained. One clone, 2b-Apr5, which is used for the subsequent procedure, codes for the same amino-acid sequence as the cDNA described in Scarpati et al. Fig. 2 depicts the total sequence of the clone 2b-Apr5 with the thromboplastin amino-acid sequence deduced therefrom.

Construction of a hybrid plasmid pTF1Fc coding for thromboplastin fusion protein.

The plasmid pCD4E gamma 1 (EP 0 325 262 A2; deposited at the ATCC under the number No. 67610) is used for expression of a fusion protein composed of human CD4 receptor and human IgG1. The DNA sequence coding for the extracellular domain of CD4 is deleted from this plasmid using the restriction enzymes HindIII and BamHI. Only partial cleavage must be carried out with the enzyme HindIII in this cas , in order to cut at only on of the two HindIII sit s c ntained in pCD4E gamma 1 (position 2198). The r sult is an opened vector in which a ukaryotic transcription r gulation s qu nc (promoter) is followed by the open HindIII sit . Th open BamHI sit is

located at th start of th coding r gions for a penta-peptide link r, followed by the hing and the CH2 and CH3 domains of human IgG1. The reading frame in th BamHI recognition s qu nc GGATCC is such that GAT is translated as aspartic acid. DNA amplification with thermostable DNA polymerase makes it possible to modify a given sequence in such a way that any desired sequences are attached at one or both ends. Two oligonucleotides able to hybridize with sequences in the 5'-untranslated region (A: 5' GATCGATTAAGCTTCGGAACCCGCTCGATCTCGCCGCC 3') or coding region (B: 5' GCATATCTGGATCCCCGTAGAATATTTCTCTGAATTCCCC 3') of thromboplastin cDNA were synthesized. Of these, oligonucleotide A is partially homologous with the sequence of the coding strand, and oligonucleotide B is partially homologous with the non-coding strand; cf. Fig. 3.

Thus, amplification results in a DNA fragment (827 bp) which contains (based on the coding strand) at the 5' end before the start of the coding sequence a HindIII site, and at the 3' end after the codon for the first three amino-acid residues of the transmembrane region a BamHI site. The reading frame in the BamHI cleavage site is such that ligation with the BamHI site in pCD4E gamma 1 results in a gene fusion with a reading frame continuous from the initiation codon of the thromboplastin cDNA to the stop codon of the heavy chain of IgG1. The desired fragment was obtained and, after treatment with HindIII and BamHI, ligated into the vector pCD4E gamma 1, as described above, which had been cut with HindIII (partially) and BamHI. The resulting plasmid was called pTF1Fc (Fig. 4).

Transfection of pTF1Fc into mammalian cells

Th fusion prot in ncod d by th plasmid pTF1Fc is call d pTF1Fc h r inaft r. pTF1Fc was transiently expr ss d in COS c lls. For this purpose, COS c lls wer

transfected with pTF1Fc with the aid of DEAE-dextran (EP A 0 325 262). Indirect immunofluorescence investigations revealed that the proportion of transfected cells was about 25 %. 24 h after transfection, the cells were transferred into serum-free medium. This cell supernatant was harvested after a further three days.

Purification of pTF1Fc fusion protein from cell culture supernatants

170 ml of supernatant from transiently transfected COS cells were collected overnight in a batch process in a column containing 0.8 ml of protein A-Sepharose at 4°C, washed with 10 volumes of washing buffer (50 mM tris buffer pH 8.6, 150 mM NaCl) and eluted in 0.5 ml fractions with eluting buffer (93:7 100 mM citric acid: 100 mM sodium citrate). The first 9 fractions were immediately neutralized with 0.1 ml of 2M tris buffer pH 8.6 in each case and then combined, and the resulting protein was transferred by three concentration/dilution cycles in an Amicon microconcentrator (Centricon 30) into TNE buffer (50 mM tris buffer pH 7.4, 50 mM NaCl, 1 mM EDTA). The pTF1Fc obtained in this way is pure by SDS-PAGE electrophoresis (U.K. Lämmli, Nature 227 (1970) 680-685). In the absence of reducing agents it behaves in the SDS-PAGE like a dimer (about 165 KDa).

Biological activity of purified TF1Fc in the prothrombin clotting time determination

TF1Fc fusion protein is active in low concentrations (> 50 ng/ml) in the one-stage prothrombin clotting time determination (Vinazzer, H. Gerinnungsphysiologie und Methoden im Blutgerinnungslabor (1979), Fischer Verlag Stuttgart). The clotting times achieved are comparable with the clotting times obtained with thromboplastin isolated from human placenta.

Example 2: Interleukin-4 receptor fusion proteins

Interleukin-4 (IL-4) is synthesized by T cells and was originally called B-cell growth factor because it is able to stimulate B-cell proliferation. It exerts a large number of effects on these cells. One in particular is the stimulation of synthesis of molecules of immunoglobulin subclasses IgG1 and IgE in activated B cells (Coffmann et al., Immunol. Rev., vol. 102 (1988) 5). In addition, IL-4 also regulates the proliferation and differentiation of T cells and other hemopoietic cells. It thus contributes to the regulation of allergic and other immunological reactions. IL-4 binds with high affinity to a specific receptor. The cDNA which codes for the human IL-4 receptor has been isolated (Idzerda et al., J. Exp. Med., vol. 171 (1990) 861-873). It is evident from analysis of the amino-acid sequence deduced from the cDNA sequence that the IL-4 receptor is composed of a total of 825 amino acids, with the 25 N-terminal amino acids acting as signal peptide. Mature human IL-4 receptor is composed of 800 amino acids and, like thromboplastin, has a three-domain structure: i) amino-terminal extracellular domain (207 amino acids); ii) transmembrane region (24 amino acids) and iii) cytoplasmic domain (569 amino acids). In the extracellular domain there are six potential sites for N-glycosylation (Asn-X-Thr/Ser). IL-4 receptor has homologies with human IL-6 receptor, with the β-subunit of human IL-2 receptor, with mouse erythropoietin receptor and with rat prolactin receptor (Idzerda et al., loc. cit.). Thus, like thromboplastin, it is not a member of the immunoglobulin family but is assigned together with the homologous proteins mentioned to the new family of hematopoietin receptors. Members of this family have four cysteine residues and a conserved sequence (Trp-Ser-X-Trp-Ser) in the extracellular domain located near the transmembrane region in common.

On the basis of the described function of the IL-4/IL-4

rec ptor system, th re is a possible therapeutic use of a recombinant form of th IL-4 r c ptor for suppressing IL-4-mediated immune reactions (for xampl transplant r jection reaction, autoimmune dis as s, all rgic r actions).

The amount of substance required for therapy makes it necessary to prepare such molecules by genetic engineering. Because of the straightforward purification by affinity chromatography and improved pharmacokinetic properties, according to the invention the synthesis of soluble forms of the IL-4 receptor as immunoglobulin fusion protein is particularly advantageous.

The IL-4 receptor fusion proteins are secreted by mammalian cells (for example CHO, BHK, COS cells) into the culture medium, purified by affinity chromatography on protein A-Sepharose and have, surprisingly, identical functional properties to the extracellular domain of the intact membrane-bound IL-4 receptor molecule.

Construction of a hybrid plasmid pIL-4RFc coding for IL-4 receptor fusion protein.

Cutting of the plasmid pCD4E gammal with XhoI and BamHI results in an opened vector in which the open XhoI site is located downstream from the promoter sequence. The open BamHI site is located at the start of the coding regions for a pentapeptide linker, followed by the hinge and the CH2 and CH3 domains of human IgG1. The reading frame in the BamHI recognition sequence GGATCC is such that GAT is translated as aspartic acid. DNA amplification with thermostable DNA polymerase makes it possible to modify a given sequence in such a way that any desired s qu nces can be attached at on or both ends. Two oligonucl otid s abl to hybridiz with sequ nc s in th 5'-untranslated r gion
(A: 5' GATCCAGTACTCGAGAGAGAAGCCGGGCGTGGTGGCTCATGC 3') or coding r gion

(B: 5' CTATGACATGGATCCTGCTCGAAGGGCTCCCTGTAGGAGTTGTG 3') of th IL-4 rec ptor cDNA which is cloned in the vector pDC302/T22-8 (Idzerda t al., loc. cit.) were synthesized. Of these, oligonucleotide A is partially homologous with the sequence of the coding strand, and oligonucleotide B is partially homologous with the non-coding strand; cf. Fig. 5. Amplification using thermostable DNA polymerase results in a DNA fragment (836 bp) which, based on the coding strand, contains at the 5' end before the start of the coding sequence an XhoI site, and at the 3' end before the last codon of the extracellular domain a BamHI site. The reading frame in the BamHI cleavage site is such that ligation with the BamHI site in pCD4E gamma 1 results in a gene fusion with a reading frame continuous from the initiation codon of the IL-4 receptor cDNA to the stop codon of the heavy chain of IgG1. The desired fragment was obtained and, after treatment with XhoI and BamHI, ligated into the vector pCD4E gamma 1, described above, which had been cut with XhoI/BamHI. The resulting plasmid was called pIL4RFc (Fig. 6).

Transfection of pIL4RFc into mammalian cells

The fusion protein encoded by the plasmid pIL4RFc is called pIL4RFc hereinafter. pIL4RFc was transiently expressed in COS cells. For this purpose, COS cells were transfected with pIL4RFc with the aid of DEAE-dextran (EP A 0 325 262). Indirect immunofluorescence investigations revealed that the proportion of transfected cells was about 25 %. 24 h after transfection, the cells were transferred into serum-free medium. This cell supernatant was harvested after a further three days.

Purification of IL4RFc fusion protein from cell culture supernatants

500 ml of supernatant from transiently transfected COS

cells were collected overnight in a batch process in a column containing 1.6 ml of protein A-Sepharose at 4°C, washed with 10 volumes of washing buffer (50 mM tris buffer pH 8.6, 150 mM NaCl) and eluted in 0.5 ml fractions with eluting buffer (93:7 100 mM citric acid: 100 mM sodium citrate). The first 9 fractions were immediately neutralized with 0.1 ml of 2M tris buffer pH 8.6 in each case and then combined, and the resulting protein was transferred by three concentration/dilution cycles in an Amicon microconcentrator (Centricon 30) into TNE buffer (50 mM tris buffer pH 7.4, 50 mM NaCl, 1 mM EDTA). The IL4RFc obtained in this way is pure by SDS-PAGE electrophoresis (U.K. Lämmli, Nature 227 (1970) 680-685). In the absence of reducing agents it behaves in the SDS-PAGE like a dimer (about 150 KDa).

Biological activity of purified IL4RFc

IL4RFc proteins binds ^{125}I-radiolabeled IL-4 with the same affinity (K_D=0.5 nM) as membrane-bound intact IL-4 receptor. It inhibits the proliferation of IL-4-dependent cell line CTLLHuIL-4RI clone D (Idzerda et al., loc. cit.) in concentrations of 10-1000 ng/ml. In addition, it is outstandingly suitable for developing IL-4 binding assays because it can be bound via its Fc part to microtiter plates previously coated with, for example, rabbit anti-human IgG, and in this form likewise binds its ligands with high affinity.

Example 3: Erythropoietin fusion proteins

Mature erythropoietin (EPO) is a glycoprotein which is composed of 166 amino acids and is essential for the development of erythrocytes. It stimulates the maturation and the terminal differentiation of erythroid precursor cells. The cDNA for human EPO has been cloned (EP-A-0 267 678) and codes for the 166 amino acids of mature EPO and a signal peptide of 22 amino acids which is essential for secretion. The cDNA can be used to

prepare recombinant functional EPO in genetically manipulated mammalian cells and the EPO can be employed clinically for the therapy of anemic manifestations of various etiologies (for example associated with acute renal failure).

Because of the straightforward purification and the improved pharmacokinetic properties, according to the invention synthesis of EPO as immunoglobulin fusion protein is particularly advantageous.

Construction of a hybrid plasmid pEPOFc coding for erythropoietin fusion protein.

This construction was carried out in analogy to that described in Example 2 (section: "Construction of a hybrid plasmid pIL-4RFc coding for IL-4 receptor fusion protein"). Two oligonucleotides able to hybridize with sequences in the vicinity of the initiation codon
(A: 5'GATCGATCTCGAGATGGGGGTGCACGAATGTCCTGCCTGGCTGTGG 3')
and of the stop codon
(B: 5' CTGGAATCGGATCCCCTGTCCTGCAGGCCTCCCCTGTGTACAGC 3')
of the EPO cDNA cloned in the vector pCES (EP-A 0 267 678) were synthesized. Of these, oligonucleotide A is partially homologous with the sequence of the coding strand, and oligonucleotide B is partially homologous with the non-coding strand; cf. Fig. 7. Amplification with thermostable DNA polymerase results in a DNA fragment (598 bp) which, based on the coding strand, contains at the 5' end in front of the initiation codon an XhoI site and in which at the 3' end the codon for the penultimate C-terminal amino acid residue of the EPO (Asp) is present in a BamHI recognition sequence. The reading frame in the BamHI cleavage site is such that ligation with the BamHI site in pCD4E gamma 1 results in a gene fusion with a reading frame continuous from the initiation codon of EPO cDNA to the stop codon of the heavy chain of IgG1. The desired fragment was obtained and, after treatment with XhoI and BamHI, ligated into

th v ctor pCD4E gamma 1, described abov , which had be n cut with XhoI/BamHI. Th resulting plasmid was call d pEPOFc (Fig. 8).

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A soluble fusion protein composed of human proteins not belonging to the immunoglobulin family, or of parts thereof, and of various portions of immunoglobulin molecules of all subclasses.

2. A fusion protein as claimed in claim 1, wherein the immunoglobulin portion is the constant part of the heavy chain of human IgG.

3. A fusion protein as claimed in claim 2, wherein the immunoglobulin portion is the constant part of the heavy chain of human IgG1 or a protein A-binding fragment thereof.

4. A fusion protein as claimed in claim 2 or claim 3, wherein the fusion takes place at the hinge region.

5. A fusion protein as claimed in claims 1 - 4, wherein the protein fused to immunoglobulin is the extracellular portion of a membrane protein or parts thereof.

6. A fusion protein as claimed in claims 1 - 4, wherein the protein fused to immunoglobulin is the extracellular portion of thromboplastin or parts thereof.

7. A fusion protein as claimed in claims 1 - 4, wherein the protein fused to immunoglobulin is the extracellular portion of a cytokine receptor or growth factor receptor or parts thereof.

8. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of IL-4 receptor or parts thereof.

9. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of IL-7 receptor or parts thereof.

10. A fusion prot in as claim d in claim 7, wher in th prot in fused to immunoglobulin is the xtrac llular portion of tumor necrosis factor r c ptor or parts thereof.

11. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of G-CSF receptor or parts thereof.

12. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of GM-CSF receptor or parts thereof.

13. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of erythropoietin receptor or parts thereof.

14. A fusion protein as claimed in claims 1 - 4, wherein the protein fused to immunoglobulin is a non-membrane-bound soluble protein or part thereof.

15. A fusion protein as claimed in claim 14, wherein the protein fused to immunoglobulin is a cytokine or growth factor or part thereof.

16. A fusion protein as claimed in claim 15, wherein the protein fused to immunoglobulin is erythropoietin or part thereof.

17. A fusion protein as claimed in claim 15, wherein the protein fused to immunoglobulin is GM-CSF or G-CSF or part thereof.

18. A fusion protein as claimed in claim 15, wher in th prot in fused to immunoglobulin is interl ukin IL-1 to IL-8 or part th reof.

19. A fusion protein as claimed in any of preceding claims 1-18, wherein a factor Xa cleavage site is additionally inserted between the immunoglobulin part and the non-immunoglobulin part.

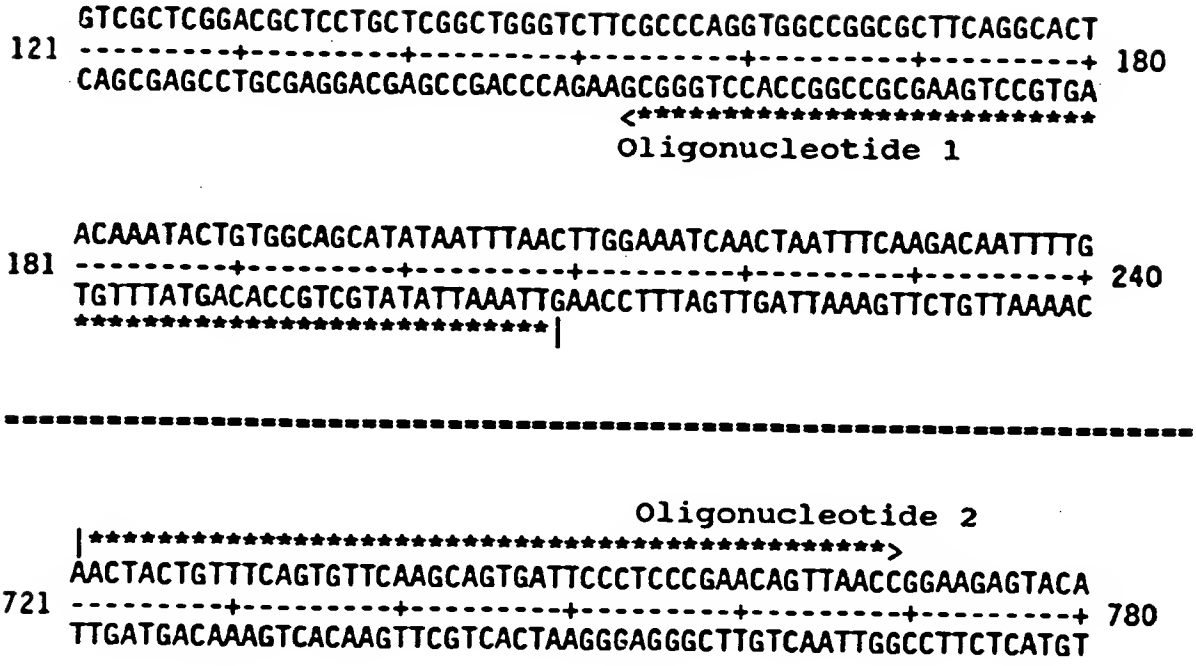
20. A process for preparing fusion proteins as claimed in any of claims 1 - 19, which comprises introducing the DNA coding for these constructs into a mammalian cell expression system and, after expression, purifying the produced fusion protein by affinity chromatography via the immunoglobulin portion.

21. The use of the fusion proteins as claimed in any of claims 1 - 19 for diagnosis.

22. The use of the fusion proteins as claimed in any of claims 1 - 19 for therapy.

23. The fusion protein as claimed in claim 1 and substantially as described h rein.

Fig. 1



By: Rogers, Bowden & Parr

Fig. 2

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       10                 30                 50
GCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTCTCGGCGAACCCC

       70                 90                 110
CTCGCACTCCCTCTGGCCGGCCCAGGGCGCCTTCAGCCCAACCTCCCCAGCCCCACGGGC

      130                150                170
GCCACGGAACCCGCTCGATCTCGCCGCCAACTGGTAGACATGGAGACCCCTGCCTGGCCC
                                         MetGluThrProAlaTrpPro

      190                210                230
CGGGTCCCGCGCCCCGAGACCGCCGTCGCTCGGACGCTCCTGCTCGGCTGGGTCTTCGCC
ArgValProArgProGluThrAlaValAlaArgThrLeuLeuLeuGlyTrpValPheAla

      250                270                290
CAGGTGGCCGGCGCTTCAGGCACTACAAATACTGTGGCAGCATATAATTTAACTTGGAAA
GlnValAlaGlyAlaSerGlyThrThrAsnThrValAlaAlaTyrAsnLeuThrTrpLys

      310                330                350
TCAACTAATTTCAAGACAATTTTGGAGTGGGAACCCAAACCCGTCAATCAAGTCTACACT
SerThrAsnPheLysThrIleLeuGluTrpGluProLysProValAsnGlnValTyrThr

      370                390                410
GTTCAAATAAGCACTAAGTCAGGAGATTGGAAAAGCAAATGCTTTTACACAACAGACACA
ValGlnIleSerThrLysSerGlyAspTrpLysSerLysCysPheTyrThrThrAspThr

      430                450                470
GAGTGTGACCTCACCGACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTC
GluCysAspLeuThrAspGluIleValLysAspValLysGlnThrTyrLeuAlaArgVal

      490                510                530
TTCTCCTACCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCTGTATGAG
PheSerTyrProAlaGlyAsnValGluSerThrGlySerAlaGlyGluProLeuTyrGlu

      550                570                590
AACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAGCCAACAATTCAGAGT
AsnSerProGluPheThrProTyrLeuGluThrAsnLeuGlyGlnProThrIleGlnSer
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By: Rogers, Bereskin & Parr

Fig. 2 (cont.)

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         610                 630                 650
TTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAGAAGATGAACGGACTTTAGTCAGA
PheGluGlnValGlyThrLysValAsnValThrValGluAspGluArgThrLeuValArg

         670                 690                 710
AGGAACAACACTTTCCTAAGCCTCCGGGATGTTTTTGGCAAGGACTTAATTTATACACTT
ArgAsnAsnThrPheLeuSerLeuArgAspValPheGlyLysAspLeuIleTyrThrLeu

         730                 750                 770
TATTATTGGAAATCTTCAAGTTCAGGAAAGAAAACAGCCAAAACAAACACTAATGAGTTT
TyrTyrTrpLysSerSerSerSerGlyLysLysThrAlaLysThrAsnThrAsnGluPhe

         790                 810                 830
TTGATTGATGTGGATAAAGGAGAAAACTACTGTTTCAGTGTTCAAGCAGTGATTCCCTCC
LeuIleAspValAspLysGlyGluAsnTyrCysPheSerValGlnAlaValIleProSer

         850                 870                 890
CGAACAGTTAACCGGAAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAGGG
ArgThrValAsnArgLysSerThrAspSerProValGluCysMetGlyGlnGluLysGly

         910                 930                 950
GAATTCAGAGAAATATTCTACATCATTGGAGCTGTGGTATTTGTGGTCATCATCCTTGTC
GluPheArgGluIlePheTyrIleIleGlyAlaValValPheValValIleIleLeuVal

         970                 990                 1010
ATCATCCTGGCTATATCTCTACACAAGTGTAGAAAGGCAGGAGTGGGGCAGAGCTGGAAG
IleIleLeuAlaIleSerLeuHisLysCysArgLysAlaGlyValGlyGlnSerTrpLys

        1030                1050                 1070
GAGAACTCCCCACTGAATGTTTCATAAAGGAAGCACTGTTGGAGCTACTGCAAATGCTAT
GluAsnSerProLeuAsnValSer

        1090                1110                 1130
ATTGCACTGTGACCGAGAACTTTTAAGAGGATAGAATACATGGAAACGCAAATGAGTATT

        1150                1170                 1190
TCGGAGCATGAAGACCCTGGAGTTCAAAAAACTCTTGATATGACCTGTTATTACCATTAG
```

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Fig. 2 (cont.)

```
      1210                1230                1250
CATTCTGGTTTTGACATCAGCATTAGTCACTTTGAAATGTAACGAATGGTACTACAACCA

      1270                1290                1310
ATTCCAAGTTTTAATTTTTAACACCATGGCACCTTTTGCACATAACATGCTTTAGATTAT

      1330                1350                1370
ATATTCCGCACTTAAGGATTAACCAGGTCGTCCAAGCAAAAACAAATGGGAAAATGTCTT

      1390                1410                1430
AAAAAATCCTGGGTGGACTTTTGAAAAGCTTTTTTTTTTTTTTTTTTTTGAGACGGAGTC

      1450                1470                1490
TTGCTCTGTTGCCCAGGCTGGAGTGCAGTAGCACGATCTCGGCTCACTTGCACCCTCCGT

      1510                1530                1550
CTCTCGGGTTCAAGCAATTGTCTGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGTGCGC

      1570                1590                1610
ACTACCACGCCAAGCTAATTTTTGTATTTTTTAGTAGAGATGGGGTTTCACCATCTTGGC

      1630                1650                1670
CAGGCTGGTCTTGAATTCCTGACCTCAGTGATCCACCCACCTTGGCCTCCCAAAGATGCT

      1690                1710                1730
AGTATTATGGGCGTGAACCACCATGCCCAGCCGAAAAGCTTTTGAGGGGCTGACTTCAAT

      1750                1770                1790
CCATGTAGGAAAGTAAAATGGAAGGAAATTGGGTGCATTTCTAGGACTTTTCTAACATAT

      1810                1830                1850
GTCTATAATATAGTGTTTAGGTTCTTTTTTTTTTCAGGAATACATTTGGAAATTCAAAAC

      1870                1890                1910
AATTGGGCAAACTTTGTATTAATGTGTTAAGTGCAGGAGACATTGGTATTCTGGGCAGCT
```

Fig. 2 (cont.)

```
      1930                 1950                 1970
TCCTAATATGCTTTACAATCTGCACTTTAACTGACTTAAGTGGCATTAAACATTTGAGAG

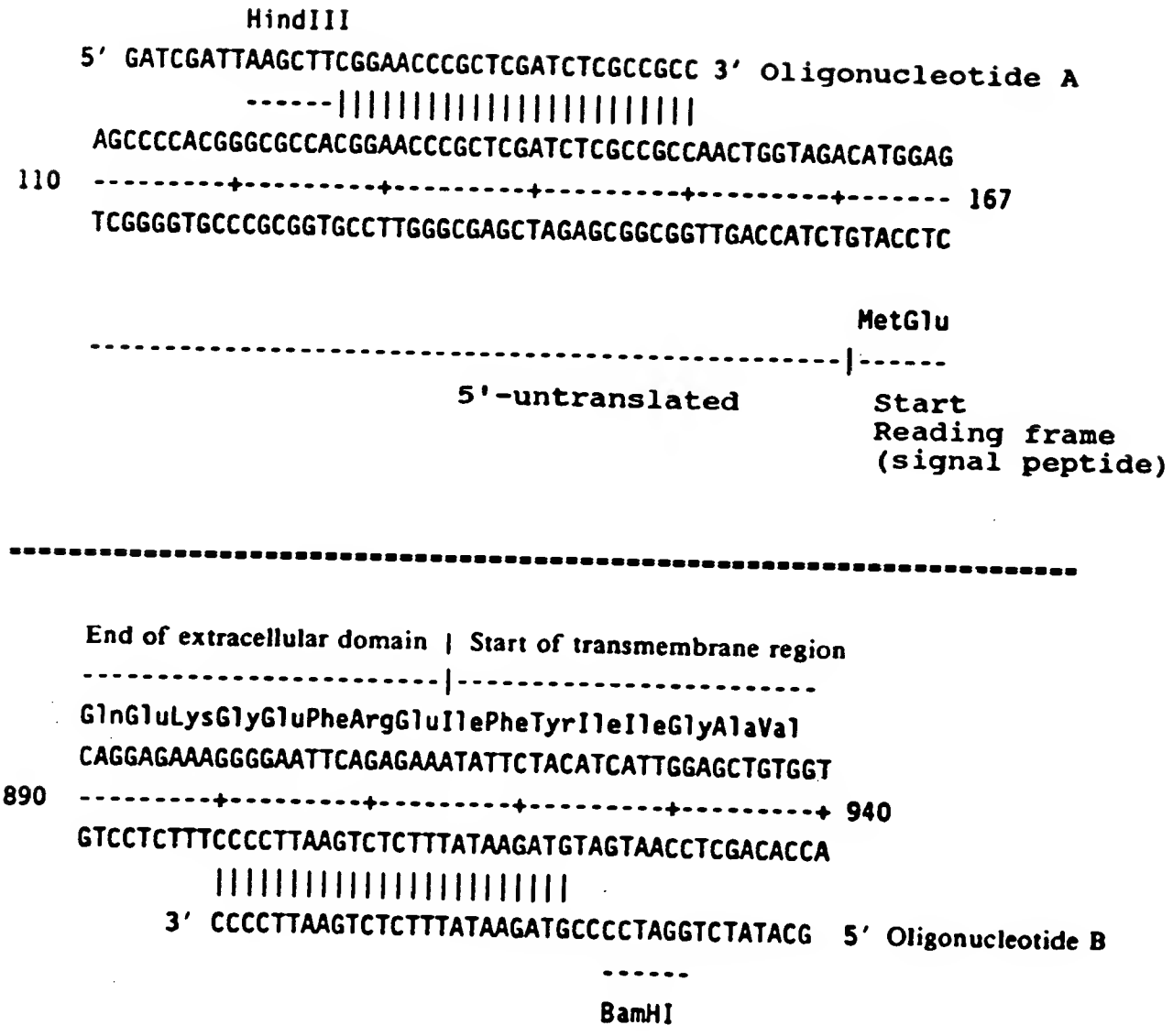
      1990                 2010                 2030
CTAACTATATTTTTATAAGACTACTATACAAACTACAGAGTTTATGATTTAAGGTACTTA

      2050                 2070                 2090
AAGCTTCTATGGTTGACATTGTATATATAATTTTTTAAAAAGGTTTTTCTATATGGGGAT

      2110                 2130                 2150
TTTCTATTTATGTAGGTAATATTGTTCTATTTGTATATATTGAGATAATTTATTTAATAT

      2170
ACTTTAAATAAAGGTGACTGGGAATTGTT
```

Fig. 3


HindIII
5' GATCGATTAAGCTTCGGAACCCGCTCGATCTCGCCGCC 3' Oligonucleotide A
AGCCCCACGGGCGCCACGGAACCCGCTCGATCTCGCCGCCAACTGGTAGACATGGAG
110
167
TCGGGGTGCCCGCGGTGCCTTGGGCGAGCTAGAGCGGCGGTTGACCATCTGTACCTC
MetGlu
5'-untranslated
Start
Reading frame
(signal peptide)
End of extracellular domain | Start of transmembrane region
GlnGluLysGlyGluPheArgGluIlePheTyrIleIleGlyAlaVal
CAGGAGAAAGGGGAATTCAGAGAAATATTCTACATCATTGGAGCTGTGGT
890
940
GTCCTCTTTCCCCTTAAGTCTCTTTATAAGATGTAGTAACCTCGACACCA
3' CCCCTTAAGTCTCTTTATAAGATGCCCCTAGGTCTATACG 5' Oligonucleotide B
BamHI

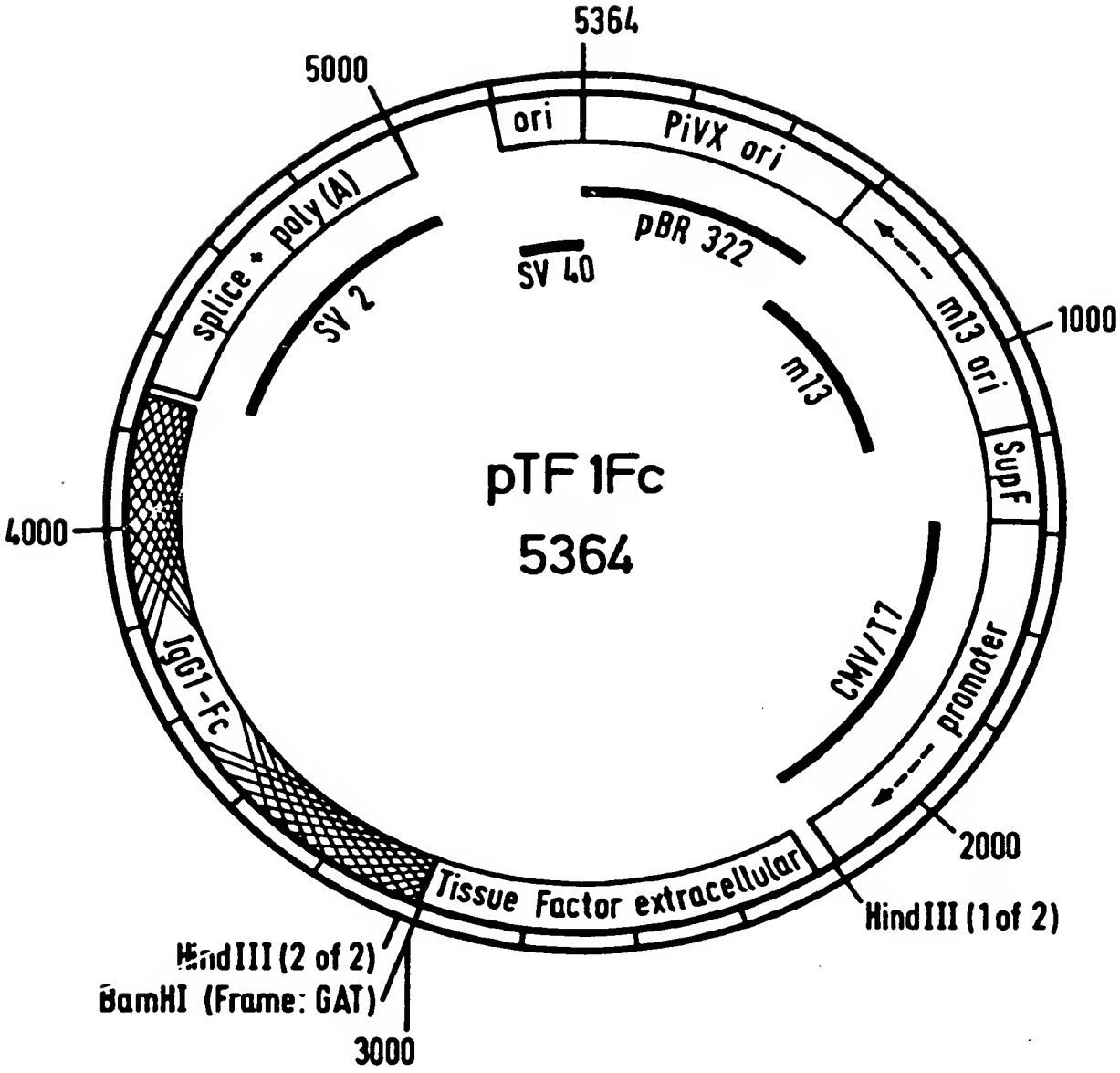
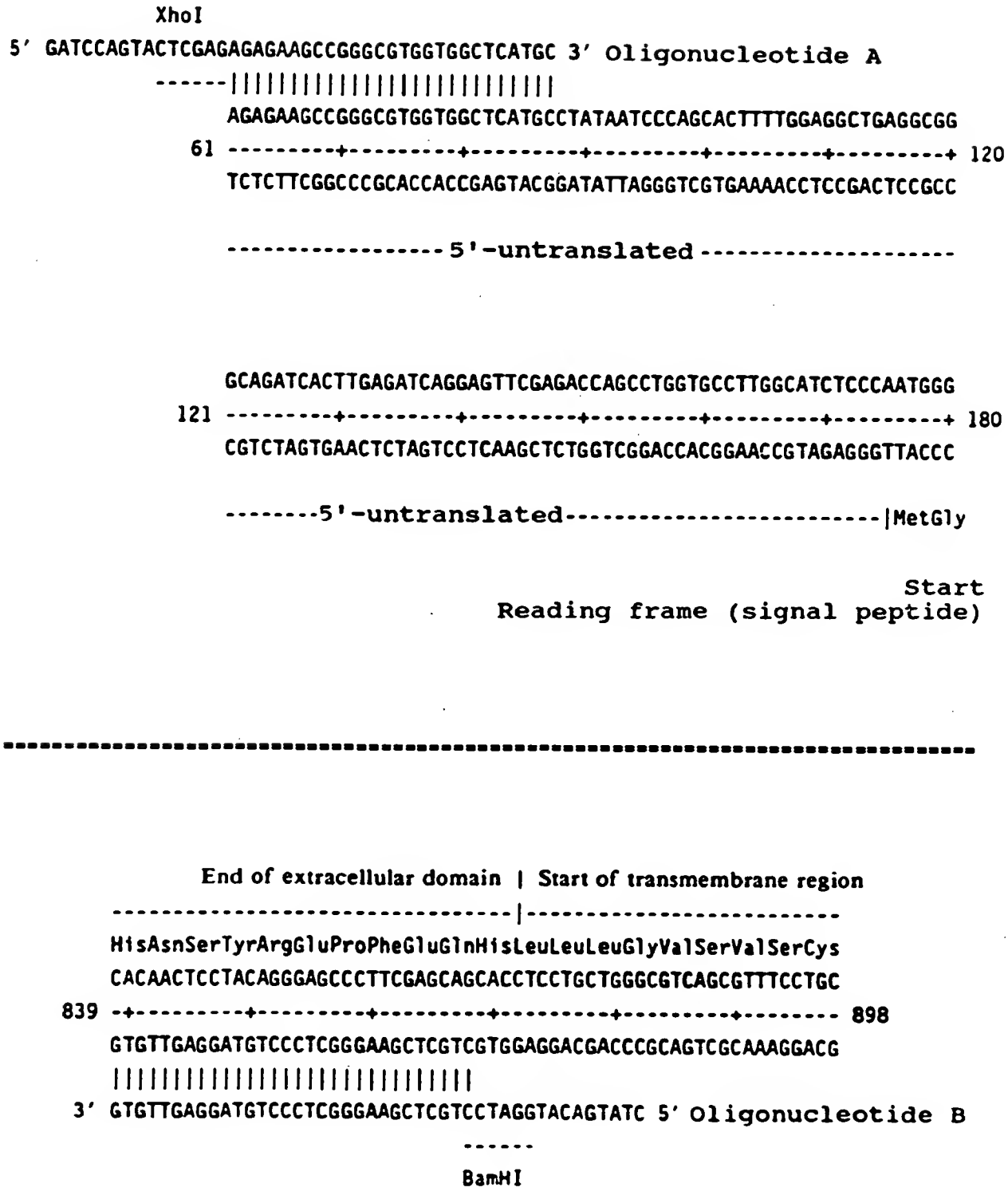

5364
5000
ori
PiVX ori
pBR 322
SV 40
SV 2
splice + poly(A)
m13
m13 ori
1000
pTF 1Fc
5364
SupF
4000
CMV/T7
promoter
IgG1-Fc
2000
Tissue Factor extracellular
HindIII (1 of 2)
HindIII (2 of 2)
BamHI (Frame: GAT)
3000

Fig. 4

Fig. 5


XhoI
5' GATCCAGTACTCGAGAGAGAAGCCGGGCGTGGTGGCTCATGC 3' Oligonucleotide A
AGAGAAGCCGGGCGTGGTGGCTCATGCCTATAATCCCAGCACTTTTGGAGGCTGAGGCGG
61
TCTCTTCGGCCCGCACCACCGAGTACGGATATTAGGGTCGTGAAAACCTCCGACTCCGCC
120
5'-untranslated
GCAGATCACTTGAGATCAGGAGTTCGAGACCAGCCTGGTGCCTTGGCATCTCCCAATGGG
121
CGTCTAGTGAACTCTAGTCCTCAAGCTCTGGTCGGACCACGGAACCGTAGAGGGTTACCC
180
5'-untranslated
MetGly
Start
Reading frame (signal peptide)
End of extracellular domain
Start of transmembrane region
HisAsnSerTyrArgGluProPheGluGlnHisLeuLeuLeuGlyValSerValSerCys
CACAACTCCTACAGGGAGCCCTTCGAGCAGCACCTCCTGCTGGGCGTCAGCGTTTCCTGC
839
GTGTTGAGGATGTCCCTCGGGAAGCTCGTCGTGGAGGACGACCCGCAGTCGCAAAGGACG
898
3' GTGTTGAGGATGTCCCTCGGGAAGCTCGTCCTAGGTACAGTATC 5' Oligonucleotide B
BamHI

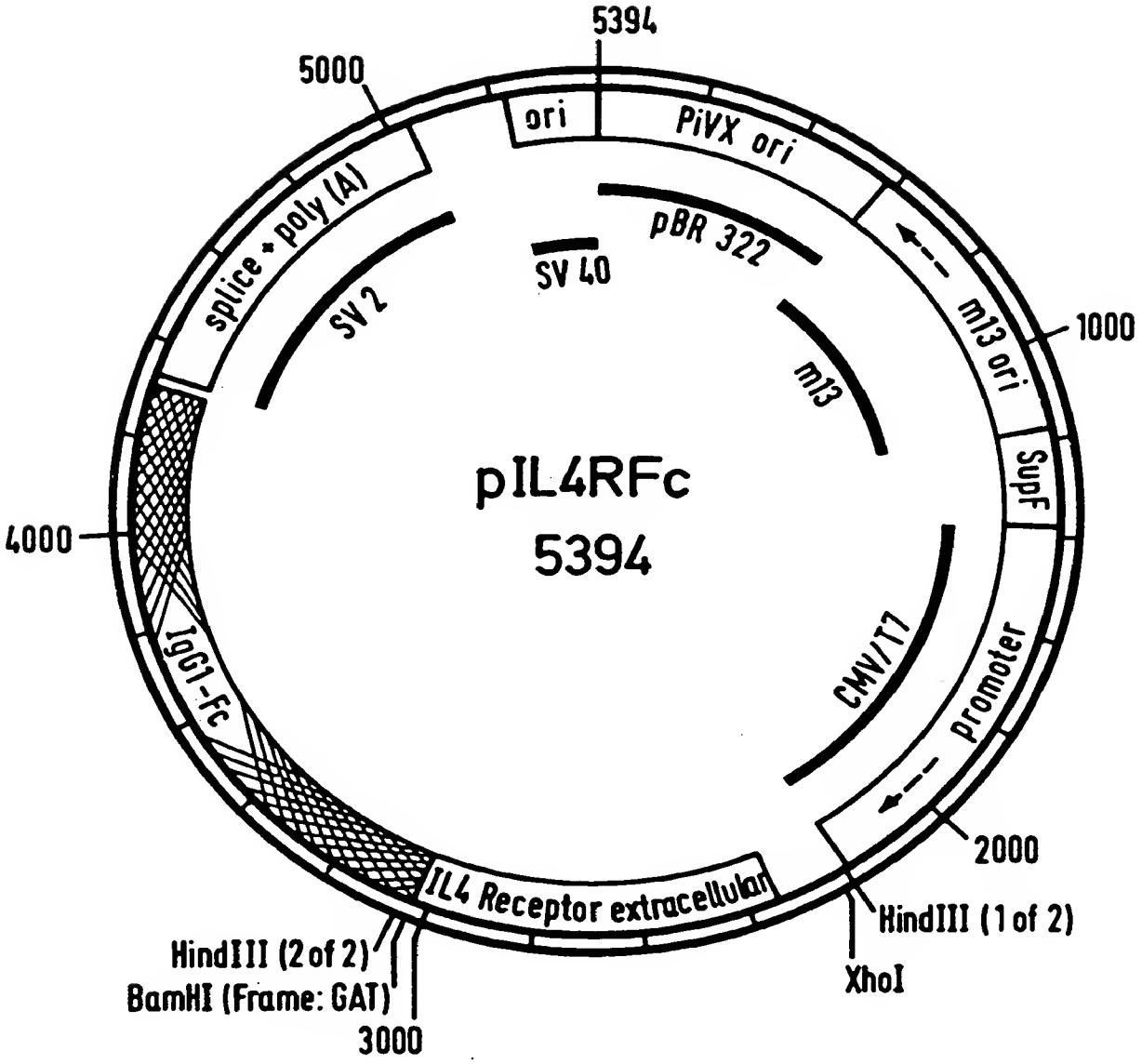

5394
5000
1000
4000
2000
3000
ori
PiVX ori
pBR 322
SV 40
SV 2
splice + poly (A)
m13
m13 ori
SupF
pIL4RFc
5394
CMV/T7
Promoter
IgG1-Fc
IL4 Receptor extracellular
HindIII (1 of 2)
XhoI
HindIII (2 of 2)
BamHI (Frame: GAT)

Fig. 6

Fig. 7

```
        XhoI
5' GATCGATCTCGAGATGGGGGTGCACGAATGTCCTGCCTGGCTGTGG 3' Oligonucleotide A
        ------||||||||||||||||||||||||||||||||
              ATGGGGGTGCACGAATGTCCTGCCTGGCTGTGGCTTCTCCTGTCCCTGCTGTCG
          182 --------+---------+---------+---------+---------+----- 235
              TACCCCCACGTGCTTACAGGACGGACCGACACCGAAGAGGACAGGGACGACAGC

              MetGlyValHisGluCysProAlaTrpLeuTrpLeuLeuLeuSerLeuLeuSer -

              Start reading frame (signal peptide)
```

```
     End of reading frame--------------|
      LeuTyrThrGlyGluAlaCysArgThrGlyAspArgEnd
      -----------------------------------|
     GCTGTACACAGGGGAGGCCTGCAGGACAGGGGACAGATGACCAGGTGTGTCCACCTGGGC
 724 ------+---------+---------+---------+---------+---------+--- 783
     CGACATGTGTCCCCTCCGGACGTCCTGTCCCCTGTCTACTGGTCCACACAGGTGGACCCG
     |||||||||||||||||||||||||||||||||
  3' CGACATGTGTCCCCTCCGGACGTCCTGTCCCCTAGGCTAAGGTC 5' Oligonucleotide B
                                   ------
                                   BamHI
```

By [illegible]

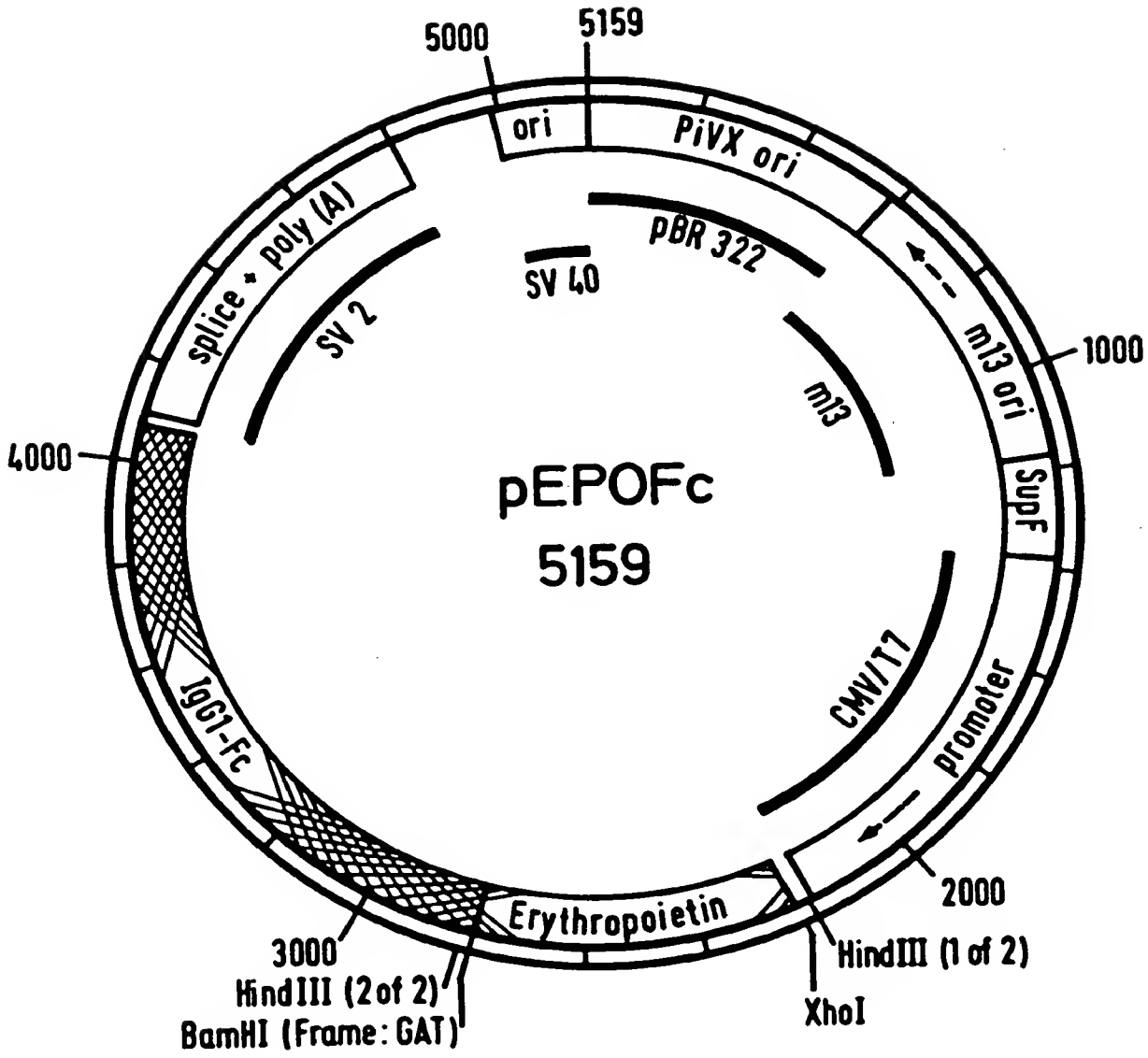

5000
5159
ori
PiVX ori
pBR 322
SV 40
SV 2
splice + poly (A)
m13
m13 ori
1000
4000
pEPOFc
5159
SupF
CMV/T7
promoter
IgG1-Fc
Erythropoietin
2000
3000
HindIII (2 of 2)
BamHI (Frame: GAT)
HindIII (1 of 2)
XhoI

Fig. 8